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SHORT COMMUNICATION

TEMPERATURE SENSITIVE GROWTH OF A HERPES TYPE VIRUS ISOLATED FROM A CHICKEN WITH MAREK'S DISEASE[1]

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Temperature sensitive (*ts*) mutants isolated from animal viruses are useful in genetic and physiological analyses of animal viruses (Fenner, 1969). The present communication briefly describes the isolation of a *ts* virus of the Biken C2 strain of a herpes-type virus (HTV) isolated by Kato et al. (1970) from a chicken with Marek's disease (MD).

The Biken C2 strain of HTV was successfully propagated in quail embryo fibroblasts (QUEF) (Onoda et al., 1970). A characteristic of this virus is that its infectivity in cell culture is largely associated with cells. The virus was assayed by a plaque method utilizing 0.9% agar overlay. Cultures of QUEF were maintained in Eagle's minimum essential medium (MEM) with 5% calf serum and 10% trytose phosphate broth. Monolayers in dishes were inoculated with HTV-infected cells. After incubation at 36 C for 24 hr most cells had become infected. Then the growth medium was replaced by medium containing nitrosoguanidine (NG) as a mutagen. This drug has been used extensively as a mutagen for DNA type viruses (Tegtmeyer et al., 1970). The infected cells were exposed to various concentrations of NG (5, 10, 20 and 40 μg/ml) at 36 C for 5 days. NG at a concentration of 40 μg/ml caused cell degeneration. Therefore it was used at a concentration of 20 μg/ml. For screening of potential viruses, the permissive and nonpermissive temperatures used were 36 C and 41 C, respectively. After treatment with NG HTV-infected cells with an infectivity of approximately 10 plaque forming units (PFU) per dishes were plated onto QUEF and kept at the permissive temperature (36 C) for 4 days. Then the plaques were carefully picked out and were each seeded in dishes containing fresh, normal QUEF monolayers. They were transferred 2 to 3 times at intervals of 4 days at 36 C. Potential viruses were then screened in QUEF at permissive and nonpermissive temperatures, using 4-fold dilutions and 2 dishes for each dilution. Viruses which showed some reduction in growth at 41 C were purified twice by plaque transfer at 36 C. Viruses harvested from single plaques of this type were tested for their abilities to multiply at 36 C and 41 C. Of 120 plaques isolated, only one was strongly suppressed by the high

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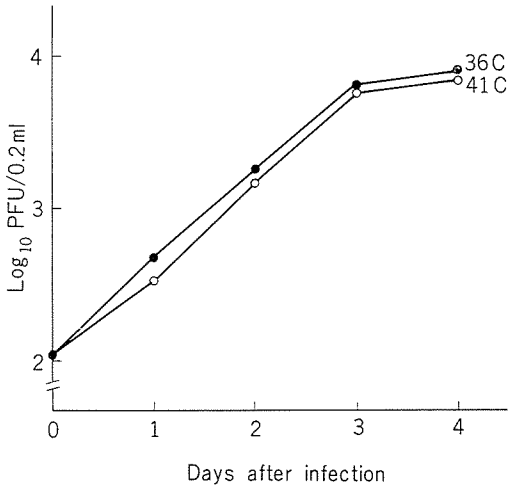

FIGURE 1. *Growth curve of parental herpes type virus (HTV). QUEF monolayers infected with HTV (1.1 × 10² PFU) were incubated at 36 C or 41 C. Samples were removed at intervals. Volumes of 0.2 ml of serial 4-fold dilutions of a suspension of infected cells were inoculated into 5.0 ml of overlay medium on each culture. Plaques were counted 6 days after infection.*

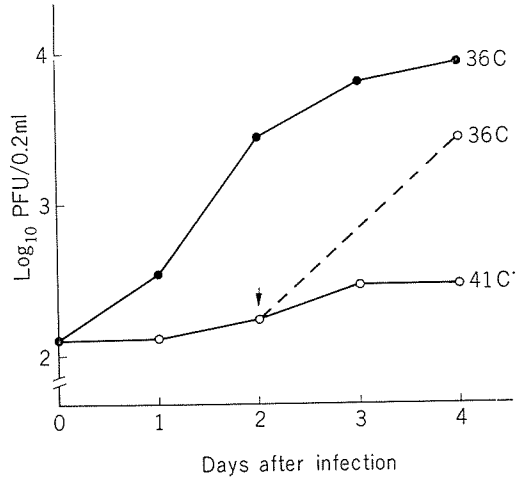

FIGURE 2. *Growth curve and temperature shift-down experiment on ts HTV. Conditions were the same as for Fig. 1. In the temperature shift-down experiment, 3 sets of QUEF cultures were infected with* ts *HTV (1.3× 10² PFU). One set of cultures was incubated at 36 C and two sets of them were incubated at 41 C. One of the latter sets was shifted to 36 C 2 days after infection and incubated for 2 days (--○--). Plaque assays were done by the same way as for Fig. 1.*

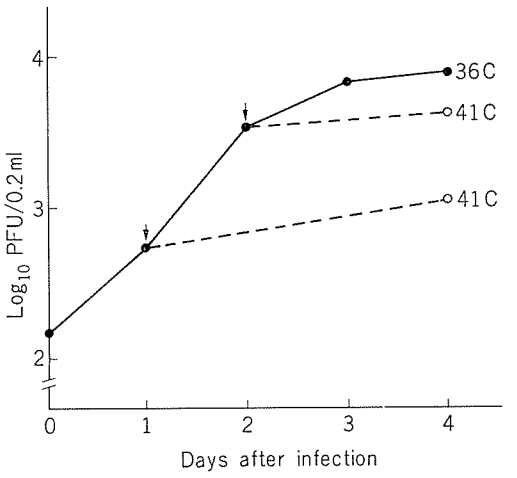

FIGURE 3. *Temperature shift-up experiment on ts HTV. Three sets of QUEF cultures were infected with ts HTV (1.5× 10² PFU) and incubated at 36 C. Two sets of them were transferred to 41 C 1 and 2 days after infection respectively and incubated until 4 days after infection (--○--). Plaque assays were done by the same way as for Fig. 1.*

temperature when compared with the parent strain. Virus titration was carried out as follows. Monolayer cultures of QUEF in 60 mm Falcon plastic dishes were infected with the parental HTV and *ts* HTV, respectively. At various times after infection, pairs of cultures were removed from the incubator, treated with trypsin and pooled. Volumes of 0.2 ml of serial 4-fold dilutions of a suspension of the cultured cells were used for inoculation. Plaque counts are made on four plates. The parent HTV grew equally well at 36 C and 41 C (Fig. 1). Growth of *ts* HTV resembled that of the parent strain at 36C, but was hardly observed at 41 C (Fig. 2). However, growth of *ts* HTV occurred when infected cultures were shifted from 41 C to 36 C 2 days after infection, as shown in Fig. 2. On the other hand, when cultures infected with *ts* HTV were transferred from 36 C to 40 C at intervals after infection, scarcely any growth occurred after the tranfer (Fig. 3). QUEF cultures infected with *ts* HTV were also ex-

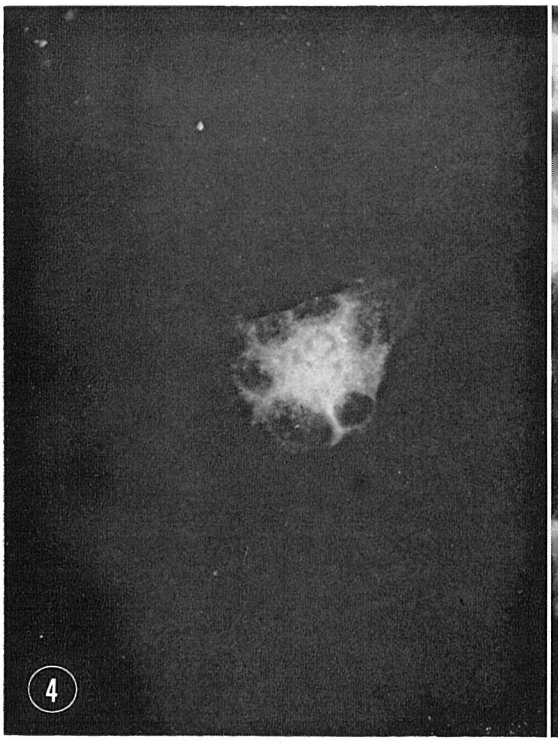

FIGURE 4. *Fluorescence photomicrograph of QUEF infected with* ts *HTV for 4 days at 41 C.*

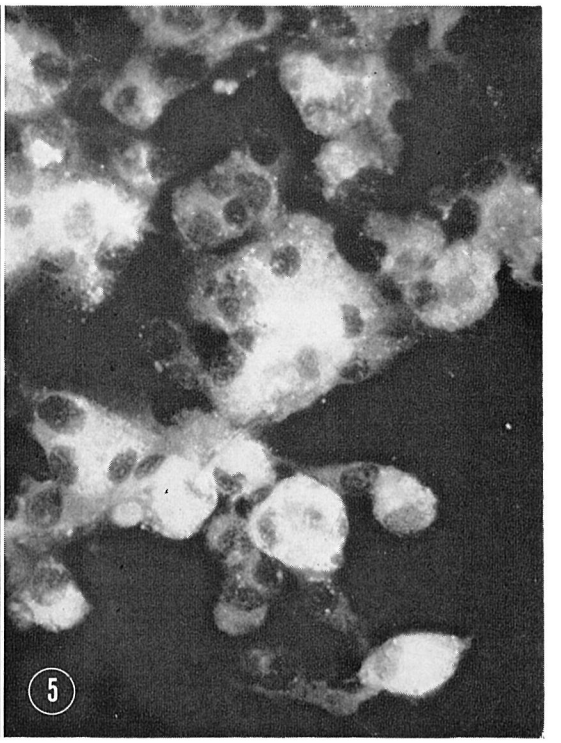

FIGURE 5. *Fluorescence photomicrograph of QUEF infected with* ts *HTV for 4 days at 36 C.*

amined by the fluorescent antibody technique (FAT). Monolayers of QUEF on coverslips were inoculated with *ts* HTV and the parental HTV. The coverslips were removed at intervals, fixed in cold acetone and stained by the direct FAT method (Naito et al., 1969). When QUEF cultures infected with *ts* HTV were incubated at the nonpermissive temperature for 4 days, scarcely any fluorescent cells were detected in them (Fig. 4). However, when cells infected with *ts* HTV were incubated for 4 days at the permissive temperature, many plaques consisting of numerous fluorescent cells were observed in them (Fig. 5). On the other hand, when cultures infected with the parental HTV were incubated for 4 days at the permissive and nonpermissive temperatures, many plaques consisting of numerous fluorescent cells were observed in both cultures. When cultures infected with *ts* HTV, were transferred from 41C to 36C or from 36C to 41C at intervals increase in the number of fluorescent cells was parallel with the growth at these permissive and nonpermissive temperatures. The morphological characteristics of plaques of *ts* HTV in duck embryo fibroblasts and QUEF were examined at 36C and found to be the same as those of the parental HTV (Onoda et al., 1970).

ts HTV seems to be a kind of mutant, but it is unknown whether its appearance is related with NG treatment, or it is a spontaneous phenomenon.

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